

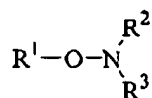
Serial No. 09/760,205

CLAIMS LISTING 030424

RDID 0013 US

What is claimed is:

18. (previously amended) An aqueous solution comprising a hydrogen-accepting coenzyme selected from the group consisting of NAD, NADP and derivatives thereof and one or more compounds selected from the group consisting of organic compounds or salts thereof having a pKa value between 1.5 and 6.0 and nitrogen compounds of the formula



in which R<sup>1</sup>, R<sup>2</sup> and R<sup>3</sup> are the same or different and denote hydrogen or a saturated or unsaturated alkyl or aryl group, the solution characterized by the absence of glucose-6-phosphate dehydrogenase.

19. (original) The solution of claim 18 wherein the organic compound is citric acid or a citrate salt.
20. (original) The solution of claim 19 wherein the concentration of the citric acid or citrate salt is about 5 to 200 mM.
21. (original) The solution of claim 18 wherein the pH is between 1.0 and 7.0.
22. (original) The solution of claim 18 wherein the nitrogen compound is a hydroxylamine derivative or salt thereof.
23. (original) The solution of claim 22 wherein the concentration of the hydroxylamine derivative or salt is between about 2 and 300 mM.
24. (original) The solution of claim 18 further comprising a boric acid derivative.

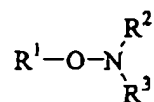
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25. (original) The solution of claim 24 wherein the concentration of the boric acid derivative is about 50 to 200 mM.
26. (original) A method for determining the concentration of a hydrogen-transferring substrate in a sample comprising:
- (a) forming a reaction mixture by combining the sample with a hydrogen-transferring enzyme for the substrate, a hydrogen-accepting coenzyme selected from the group consisting of NAD, NADP and derivatives thereof, and one or more compounds selected from the group consisting of organic compounds or salts thereof having a pKa value between 1.5 and 6.0 and nitrogen compounds of the formula
$$\text{R}^1-\text{O}-\overset{\text{R}^2}{\underset{\text{R}^3}{\text{N}}}$$
in which R<sup>1</sup>, R<sup>2</sup> and R<sup>3</sup> are the same or different and denote hydrogen or a saturated or unsaturated alkyl or aryl group, the reaction mixture characterized by the absence of glucose-6-phosphate dehydrogenase, and
  - (b) detecting the change in absorbance of the coenzyme as a measure of the concentration of the substrate present in the sample.
27. (original) A method for determining the activity of a hydrogen-transferring enzyme in a sample comprising:
- (a) forming a reaction mixture by combining the sample with a hydrogen-transferring substrate for the enzyme, a hydrogen-accepting coenzyme selected from the group consisting of NAD, NADP and derivatives thereof, and one or more compounds selected from the group consisting of

organic compounds or salts thereof having a pKa value between 1.5 and 6.0 and nitrogen compounds of the formula



in which R<sup>1</sup>, R<sup>2</sup> and R<sup>3</sup> are the same or different and denote hydrogen or a saturated or unsaturated alkyl or aryl group, the reaction mixture characterized by the absence of glucose-6-phosphate dehydrogenase, and

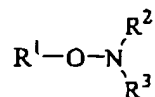
- (b) detecting the change in absorbance of the coenzyme as a measure of the activity of the enzyme present in the sample.
28. (original) The method of claim 26 wherein the hydrogen-transferring substrate is selected from the group consisting of lactate, glutamate, ammonia, alcohol, glyceraldehyde-3-phosphate and glucose.
29. (original) The method of claim 27 wherein the enzyme is selected from the group consisting of dehydrogenases of lactate, glutamate, alcohol, glycerol-3-phosphate and glucose.
30. (original) The method of claim 26 or 27 wherein the organic compound is citric acid or a citrate salt.
31. (original) The method of claim 30 wherein the concentration of the citric acid or citrate salt is about 5 to 200 mM.
32. (original) The method of claim 26 or 27 wherein the pH of the reaction mixture is between about 8.5 and 10.0.

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33. (original) The method of claim 26 or 27 wherein the nitrogen compound is a hydroxylamine derivative or salt thereof.
34. (original) The method of claim 33 wherein the concentration of the hydroxylamine derivative or salt is between about 2 and 300 mM.
35. (original) The method of claim 26 or 27 wherein the reaction mixture further comprises a boric acid derivative.
36. (original) The method of claim 35 wherein the concentration of the boric acid derivative is about 50 to 200 mM.
37. (original) A kit for determining the concentration of a hydrogen-transferring substrate in a sample comprising:
- (a) a first reagent comprising a hydrogen-transferring enzyme for the substrate in a buffer having a pH between about 8.5 and 10.0 and
  - (b) a second reagent comprising a hydrogen-accepting coenzyme selected from the group consisting of NAD, NADP and derivatives thereof and one or more compounds selected from the group consisting of organic compounds or salts thereof having a pKa value between 1.5 and 6.0 and nitrogen compounds of the formula



in which R<sup>1</sup>, R<sup>2</sup> and R<sup>3</sup> are the same or different and denote hydrogen or a saturated or unsaturated alkyl or aryl group, the second reagent characterized by the absence of glucose-6-phosphate dehydrogenase.

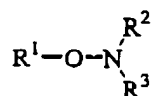
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38. (original) A kit for determining the activity of a hydrogen-transferring enzyme in a sample comprising:

- (a) a first reagent comprising a hydrogen-transferring substrate for the enzyme and a buffer having a pH between about 8.5 and 10.0 and
- (b) a second reagent comprising a hydrogen-accepting coenzyme selected from the group consisting of NAD, NADP and derivatives thereof and one or more compounds selected from the group consisting of organic compounds or salts thereof having a pKa value between 1.5 and 6.0 and nitrogen compounds of the formula



in which R<sup>1</sup>, R<sup>2</sup> and R<sup>3</sup> are the same or different and denote hydrogen or a saturated or unsaturated alkyl or aryl group, the second reagent characterized by the absence of glucose-6-phosphate dehydrogenase.

- 39. (original) The kit of claim 37 or 38 wherein the organic compound is citric acid or a citrate salt.
- 40. (original) The kit of claim 39 wherein the concentration of the citric acid or citrate salt is about 5 to 200 mM.
- 41. (original) The kit of claim 37 or 38 wherein the second reagent has a pH between about 1.0 and 7.0.
- 42. (original) The kit of claim 37 or 38 wherein the nitrogen compound is a hydroxylamine derivative or salt thereof.

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43. (original) The kit of claim 42 wherein the concentration of the hydroxylamine derivative or salt is about 2 to 300 mM.
44. (original) The kit of claim 37 or 38 wherein the first reagent further comprises a boric acid derivative.
45. (original) The kit of claim 44 wherein the concentration of the boric acid derivative is about 50 to 200 mM.